Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5	reubinoff.inv. and vs1	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/08/30 13:09
L2	15	embryonic and vs1	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/08/30 13:09
L3	12	embryonic and vs1 and (freeze or vitrification or frozen or vitrify or OPS or vitrified)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/08/30 13:10

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5	reubinoff.inv. and vs1	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/08/30 13:09

[0194] Early passage cells were cryo-preserved in clumps of about 100 cells by using the open pulled straw (OPS) vitrification method (Vajta et al 1998) with some modifications. French mini-straws (250 .mu.l, IMV, L'Aigle, France) were heat-softened over a hot plate, and pulled manually until the inner diameter was reduced to about half of the original diameter. The straws were allowed to cool to room temperature and were than cut at the narrowest point with a razor blade. The straws were sterilised by gamma irradiation (15-25 K Gy). Two vitrification solutions (VS) were used. Both were based on a holding medium (HM) which included DMEM containing HEPES buffer (Gibco, without sodium pyruvate, glucose 4500 mg/L) supplemented with 20% fetal bovine serum (Hyclone, Logan, Utah). The first VS (VS1) included 10% dimethyl sulfoxide (DMSO, Sigma) and 10% ethylene glycol (EG, Sigma). The second vitrification solution (VS2) included 20% DMSO, 20% EG and 0.5M sucrose. All procedures were performed on a heating stage at 37.degree. C. 4-6 clumps of ES cells were first incubated in VS1 for 1 minute followed by incubation in VS2 for 25 seconds. They were then washed in a 20 .mu.l droplet of VS2 and placed within a droplet of 1-2 .mu.l of VS2. The clumps were loaded into the narrow end of the straw from the droplet by capillary action. The narrow end was immediately submerged into liquid nitrogen. Straws were stored in liquid nitrogen. Thawing was also performed on a heating stage at 37.degree. C. as previously described with slight modifications (Vatja et al 1998). Three seconds after removal from liquid nitrogen, the narrow end of the straw was submerged into HM supplemented with 0.2M sucrose. After 1 minute incubation the clumps were further incubated 5 minutes in HM with 0.1 M sucrose and an additional 5 minutes in HM.